Exhibit 2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| IN RE APPLICATION OF: Gerald L. Mechanic | § | DOCKET NO.: BIOC,002/CIP |
| SERIAL NO: 07/557,639 | § | GROUP ART NO.: 112 |
| FILED: July 30, 1990 | § | |
| TITLE: PROCESS FOR CROSS-LINKING COLLAGENOUS MATERIAL AND RESULTING PRODUCT | § | EXAMINER: S. Marquis |



## DECLARATION OF DAVID T. CHEUNG, PH.D.

The Honorable Commissioner
of Patents & Trademarks
Washington, D.C. 20231

Sir:

I, David T. Cheung, hereby declare as follows:

1. I hold the A.B (Chemistry, 1973, Augsburg College), M.S. (Biochemistry, 1976, University of Minnesota) and Ph.D. (Biochemistry, 1981, University of Southern California) degrees. I am currently employed by the University of Southern California of Medicine, Department of Surgery as a Research Associate Professor conducting research into the molecular organization of mammalian structural proteins such as collagen and elastin. I have previously been Research Assistant at the respective Schools of Medicine at the University of Minnesota and Southern California (1973-1980), was a post-doctoral Research Associate in the Department of Orthopaedics of the University of Southern California School of Medicine (1980-1983), and a Research Assistant Professor in the Department of Orthopaedics of the University of Southern California School of Medicine (1983-1991). Since 1990, I have been the Associate Director of the University

of Southern California Orthopaedic Hospital Laboratory of Connective Tissue Biochemistry, all as more particularly described in my resume attached hereto as Exhibit A.

2. I have conducted extensive analyses in the fields of biomaterials and bioprostheses where my experience with connective tissue structural proteins is applied. I have published extensively on these subjects, as evidenced by the list of publications in my resume (Exhibit A), and have consulted extensively to Carbomedics, Inc., a medical device and prosthesis manufacturer working on the development of certain bioimplantable prostheses including collagen as a component thereof. In this latter role, I have become familiar with a photooxidative process for the formation of cross-links between collagen fibrils invented by Gerald L. Mechanic which is described in the captioned patent application, and as I have learned about that patent application, so also have I become familiar with the Kuntz patent, U.S. Patent No. 3,152,976. I have been asked to study that patent and to help identify the differences between the process described in that patent and the process described in the captioned application, as well as the collagen product resulting from the respective processes. To do so, I have found it necessary to conduct certain experiments, and the results of the experiments are reported in this Declaration. Also set out in this Declaration is an explanation as to why the results reported in this Declaration (as compared to the results of the Kuntz patent) are important to the problem to which the present invention offers a solution.

3. Because it helps place so many other items relating to this invention in perspective, I will first address the problem to which the present invention is directed. As stated at page 4, lines 29-34 of the specification of the present application, one of the objects of the present invention

> is to provide a collagenous product, and a method of making that product, having physical-chemical properties which make that product suitable for use as a biomaterial for use as an artificial tendon, heart valve, or pericardial patch.

It happens that a material which is useful as, for instance, the valve leaflet of an artificial heart valve, would have properties that would make it suitable for many other uses as a bioprosthetic such that we can use the prosthetic heart valve as a model, or paradigm system, to which reference can be made for assessing the utility of such a biomaterial.

4. Bioprosthetic heart valves fabricated from glutaraldehyde-preserved porcine aortic valves or bovine parietal pericardium are commonly used to replace diseased human cardiac valves. *See*, for instance, L.H. Cohn, et al., "Cardiac Bioprostheses", New York: York Medical Books (1982), pp. 1-591. However, both such valves are subject to mineralization, i.e., calcification, such that they stiffen over time, with the result that their function is impaired and/or failure occurs. Re-operation is necessitated in at least 20-25% of adult patients within 7-10 years and is even more likely in children. F.J. Schoen, et al., "Bioprosthetic Heart Valve Failure: Pathology and Pathogenesis", 2 Cardiol. Clin. 717-739 (1984); S.P. Sanders, et al., "Use of Hancock porcine xenografts in children and adolescents", 46 Am. J. Cardiol. 429-438 (1980). Such materials also elicit such host reactions as fibrin deposition and immune response. D.J. Slanczka, et al., "Immunogenicity of glutaraldehyde-treated porcine heart valves", 6 IRCS Medical Science: Bio-Technology; Cardiovascular System 421 (1978). Indeed, it was partly for these reasons that I made the invention described in U.S. Patent No. 4,378,224 (M.E. Nimni and D.T. Cheung, co-inventors), directed to a "Coating for Bioprothestic Device and Method of Making Same".

5. Attempts have also been made to provide synthetic materials for such uses, urethanes being a material which has been used with modest success. *See*, for instance, U.S. Patent Nos. 4,127,124 (D.C. Clagett, et al.), 4,523,005 (M. Szycher), 4,687,831 (N. Ogata, et al.) and 4,831,065 (H. Pietsch, et al.). However, in addition to calcification, such materials are themselves subject to a number of disadvantages and limitations such as fibrin layering, aneurysm formation, and lipid deposition

such that glutaraldehyde-preserved biomaterials are used more frequently in such applications as replacement heart valves. The search for ways to improve these bioprosthetic materials continues, and the preliminary data reported in this Declaration indicates that the method of the present invention may provide a significant improvement in the performance of replacement heart valves (and other bioprostheses) fashioned from collagenous tissues.

6. An example of the results which are being obtained with the product of the process of the present invention is the data I recently obtained from an experiment in which pieces of various collagenous tissues were implanted subcutaneously in rats to test susceptibility to mineralization, antigenicity, break-down and/or resorption, and other properties relating to their ability to function as, for instance, a heart valve leaflet. This experiment is an accepted model for evaluation of the susceptibility of a bioprosthetic heart valve to calcification. See, for instance, R.J. Levy, et al., "Biologic determinants of dystrophic calcification and osteocalcin deposition in glutaraldehyde preserved porcine aortic heart leaflets implanted subcutaneously in rats," 113 Am. J. Pathol. 144 (1983). In this experiment, four groups of 10 three-month old rats were each implanted with two 6 cm x 1 cm samples of bovine pericardial tissue prepared as follows:

A. photoxidatively cross-linked in accordance with the method of the present invention using methylene green as the dye

B. preserved with 0.3% glutaraldehyde

C. untreated

D. photoxidatively cross-linked in accordance with the method of the present invention using methylene blue as the dye.

Each sample was explanted after four months and a subjective grading system was used to assess the gross morphological condition of each sample upon explant. The data can be summarized as follows:

| | | |
|---|---|---|
| A. | methylene green cross-linked | mushy - tore apart easily and looked like group C explants |
| B. | glutaraldehyde | hard capsule |
| C. | untreated | tore apart easily |
| D. | methylene blue cross-link | white, very flexible, but strong against mechanical pulling) resists stretching), looked to be in same condition as unimplanted fresh tissue |

Histological analysis bore out these gross observations - across each group, the methylene blue cross-linked tissue data was consistent with the kind of performance one would look for from an ideal biomaterial: low mineral deposition and immunogenicity, high physical strength, and minimal tissue reabsorption. These results indicate that (a) the process of the present invention yields a product which is much more stable against biodegradation than untreated tissues; (b) the product of the present method elicits much less foreign body response by the host than untreated tissues; (c) the product of the present invention retains many of its desirable original (e.g., pre-implant) properties; and (d) not all dyes are suitable for use in accordance with the method of the present invention (and some may not be effective at all).

7. In my role as a consultant to Carbomedics, Inc., I have participated on a project team which has also constructed heart valves from the product of the process of the present invention and implanted those valves in sheep. The results of that study indicate that the process of the present invention results in a product which significantly outperforms conventional bioprosthetic heart valves. In this study, valve leaflets fabricated from bovine pericardial tissue and cross-linked in accordance with the method of the present invention were attached to a stent of a type proprietary to Carbomedics, Inc. and the resulting valve implanted into three clinically healthy, mature female sheep. Four sheep also received valves having glutaraldehyde preserved leaflets in that same stent, and one was implanted with a clinically available pericardial valve

manufactured by Baxter and identified as the Carpentier-Edwards pericardial valve in which the tissue is stabilized with glutaraldehyde. The valves were explanted on the following schedule (the animals which received a valve fabricated with tissue cross-linked in accordance with the method of the present invention are denoted by the entry "photooxidatively cross-linked" in the "valve type" column):

| Sheep | Valve Type | Explanted |
|---|---|---|
| Epsilon 1 | Photooxidatively cross-linked | 5 months |
| Epsilon 3 | Glutaraldehyde preserved, Carbomedics stent | 5 months |
| Epsilon 7 | Glutaraldehyde preserved, Carbomedics stent | 5 months |
| Epsilon 9 | Carpentier-Edwards pericardial valve | 5 months |
| Epsilon 11 | Photooxidatively cross-linked | 12 months |
| Epsilon 13 | Photooxidatively cross-linked | 18 months |
| Epsilon 16 | Glutaraldehyde preserved, Carbomedics stent | * |
| Epsilon 17 | Glutaraldehyde preserved, Carbomedics stent | ** |

* Scheduled for twelve months, animal expired at five months
** Scheduled for three months, animal expired at two months

With the exception of the valve explanted from Epsilon 9, which was calcified to the point of stenosis of two of the three leaflets, all of the valves were explanted in operating condition; leaflets were soft and pliable, showed little or no calcification, and angiography performed prior to implant indicated clinically normal atrial to ventricular pressure gradients. Two of the animals which received the glutaldehyde preserved leaflets in the Carbomedics proprietary stent expired early and a third (Epsilon 7) delivered a still-born lamb during

the experiment - studies are currently being conducted which are attempting to determine whether these events were related to the valve implant. One conclusion that is clear is that the valves which included photooxidatively cross-linked tissue of the present invention performed in exemplary fashion. Indeed, to my knowledge, no other combination of valve type and/or leaflet tissue has ever provided these kinds of results. Attached hereto as Exhibit B is a photocopy of the autopsy reports for Epsilon 1, 9, 11, and 13 supporting these conclusions.

8. Having obtained data which clearly indicates that the above-quoted object of the present invention has been achieved, I turn now to the experiments which I have conducted for the purpose of identifying the differences between the process of the above-identified Kuntz patent and the process of the present invention. I am aware that the claims pending in the present application have been rejected on the basis of the Kuntz patent, and these experiments are intended to demonstrate why that rejection is inappropriate. Specifically, in the **Official Action of June 27, 1991** in the present application, it is stated (at the top of page 4) that

> No unexpected or unobvious results have been shown. Applicant is invited to submit evidence to the contrary. No unobvious or unexpected results are seen by using one form of collagenous material over another form since the amino acids [of the collagen] are the portion [of the protein] which undergo[s] cross-linking.

The evidence presented in this Declaration is intended as a response to this explicit invitation to submit evidence indicating unexpected results and which demonstrates the differences in cross-linking one form of collagen over another.

9. Before continuing with a description of the experiments, however, an aside (which also points out a difference between the process described in the Kuntz patent and the process of the present invention): the Kuntz patent is said to be directed to a method of cross-linking collagen to form articles which are said to "possess distinctively high tensile

strength and rapid in vivo absorption time [emphasis added]" (see col. 3, lines 44-47 of that patent). The present invention is directed to a method which produces a product which is used to advantage "in medical prosthetics due to its stability" because it "resists in vivo degradation...when implanted" (page 6, lines 10-13 of the specification). In other words, the product of the Kuntz process is intended to be degraded rapidly when implanted into a patient while the product of the process of the present invention is intended to be resistant to degradation for extended periods (years). As evidenced by the rat experiments summarized in paragraph 6 above, the product of the present invention is indeed stable when implanted subcutaneously over extended periods, indicating that the results are not just a difference in degree as compared to the results obtained by Kuntz. Instead, the results obtained with the method of the present invention are different in kind, e.g., they are unexpected, in light of those reported in the Kuntz patent.

10. Given what appears upon superficial review to be the substantial similarity between the method disclosed in the Kuntz patent and the present method, I was asked to help identify the differences. To do so, I first had to rule out the possibility that the results reported in the Kuntz patent were not reported accurately. Presented contemporaneously with this Declaration is the **Rule 132 Declaration of Mark A. Moore, Ph.D.**, who replicated the Kuntz process for the purpose of verifying the accuracy of the results presented in the patent. Dr. Moore (in paragraph 10 of his Declaration) concludes that the results he obtained are consistent with those set out in Kuntz.

11. Having ruled out that possibility, I have reviewed the specification of the Kuntz patent in an attempt to explain this difference in the results obtained with the method of the present invention, and have identified a very basic difference which in and of itself probably explains why the results of the present invention are the opposite of those reported in Kuntz. Specifically, Kuntz does not teach the photooxidative cross-linking of collagen fibrils (as will be set out below, it

actually does not even teach cross-linking at all in spite of its many references to cross-linking). In spite of the many references in the specification of that patent to "intact collagen fibrils", "native collagen", and "reconstituted fibrils" (and see the end of the next paragraph with respect to the use of the latter in the Kuntz patent), neither the product of the Kuntz process nor the collagenous materials used in that process are true native collagen fibrils. The following discussion will set out the basis for that statement.

12. Native collagen fibrils have a specific structure which results from the alignment of the individual collagen molecules comprising the fibril in a helix in such a way that the fibril is referred to as being a "quarter-staggered array". Reference is made to the Exhibit A attached to the **Response to Official Action of September 14, 1990** filed in the present application for additional information on the structure of collagen fibrils and their distinctive striated appearance resulting from the quarter-staggered configuration of the collagen molecules. Although it is possible that Kuntz knew of the quarter-staggered configuration of collagen fibrils in 1961 when that application was filed (the research which demonstrated the configuration was published late in 1959), the only indication given in the specification that she even recognized that there might be a difference between different collagen states is the repeated incorrect references to intact collagen fibrils as if it was recognized that such differences might exist but they either were not appreciated or understood. It is now known that only collagen in the intact, native collagen fibril plays a role as a structural/connective tissue protein and that such fibrils can only be obtained by reconstitution of collagen under precisely controlled conditions, the most important of which is that the reconstitution be performed at physiological pH (i.e., pH = 7.4). Extraction or dispersion in acid, as described in Kuntz, causes the native fibrils to unwind into collagen molecules, and no reference is made in that patent as to how the collagen was reconstituted or, in most cases, no attempt was made to

reconstitute the acid suspension of collagen into fibrils. Instead, the term "reconstitution" is used in the Kuntz patent to describe the process of re-forming the collagenous materials to a certain three-dimensional shape or form rather than the re-aggregation of collagen molecules packed in quarter-staggered array to form native-like collagen fibrils.

13. The fact that the Kuntz patent does not contemplate the cross-linking of collagen fibrils is clear from the methods described in the Kuntz patent itself. Examples III, IV, and V of that patent describe the extraction of collagen from papain (malt-distase) treated beef tendon in acid (and all other examples refer to collagen dispersions prepared as described in those two examples). It is well known (and was well known in 1961 when the Kuntz patent was filed) that acidic extraction results in soluble collagen (collagen fibrils are insoluble):

> Since the very early work of Zachariades (1900), it has been known that the extraction of native or intact collagen fibrils in the cold with dilute organic acids leads to the solubilization of undenatured collagen molecules...[emphasis added].

A. Veiz, "Intact Collagen", in G.N. Ramachandran (Ed.), Treatise on Collagen, Vol. 1, New York: Academic Press (1967), p. 379. The method referred to in this quotation is exactly the method described in Examples III, IV, and V of the Kuntz patent, such that it is clear that Kuntz was working with soluble collagen, e.g., collagen molecules, not intact, native collagen fibrils. The specification of the Kuntz patent even states (at col. 6, lines 53-55):

> The concentration of collagenous materials in the acid dispersion or aqueous solution employed as starting material ...[emphasis added].

It is therefore quite clear that not only did the Kuntz patent not contemplate the differences in the state of the collagen being used but that the Kuntz patent itself recognizes that the material which was exposed to light in accordance with that method was soluble collagen.

14. In a further attempt to identify the differences between the method of the Kuntz patent and the method of the present invention, I conducted the following experiments.

A. Experiment 1

So-called "lathyritic" rat skin was obtained by feeding immature rats $\beta$-aminopropylinitrile (BAPN); dietary BAPN prevents the formation of cross-linkages between collagen molecules in the living rat such that the skin samples show a relatively low content of insoluble (cross-linked and non-extractable) collagen. Lathyritic rat skin (BAPN rat skin) samples were treated with methylene blue as described in the method of the present invention (designated as "treated BAPN skin"), analyzed and compared with native untreated tissues (designated as "control BAPN skin"):

1. Treated BAPN skin - BAPN rat skin treated with methylene blue as described in the method of the present invention.
2. Control BAPN skin - Non-treated native BAPN rat skin.

The samples were analyzed by measuring the amounts of collagen extractable by acid (0.5 M acetic acid) and neutral-salt (1 M, pH = 7.4) solution (referring to Experiment 1, Exhibit C). Cross-linked collagen in the skin cannot be solublized by these extraction conditions whereas non-cross-linked collagen can be extracted by these solutions readily. The results showed that sample 1 (Treated BAPN skin) contained a much lower amount of extractable (non-cross-linked) collagen than sample 2 (Control BAPN skin). The extracts of the samples were also analyzed by sodium dodecylsulfate ($NaDodSO_4$) gel electrophoresis (referring to Fig. 1 of Exhibit C). The results showed that sample 1 contained much less extractable collagen than sample 2. Also, the extracts of sample 1 contained more high molecular weight cross-linked products.

B. Experiment 2

Collagen extracted from the BAPN rat skin can be reconstituted back into native fibrils under carefully controlled physiological conditions (e.g., pH = 7.4, 1 M salt solution). The reconstituted fibril samples were also treated with methylene blue as described in the method of the present invention (designated as "Treated BAPN Fibrils"), analyzed and compared with reconstituted fibril samples which were not treated by methylene blue (designated as "control BAPN Fibrils"):

3. Treated BAPN Fibrils - Reconstituted BAPN skin collagen fibrils treated with methylene blue.
4. Control BAPN Fibrils - Reconstituted BAPN skin collagen fibrils without any treatment.

The samples were analyzed by measuring the amount of collagen extractable by neutral-salt solution (referring to Experiment 2, Exhibit C). The results were very similar to Experiment 1. Treated fibrils (sample 3) were much less extractable than untreated fibrils (sample 4). The $NaDodSO_4$ gel profile of the acid extracts (or suspension in the case of sample 3) showed that treated fibrils (sample 3) contained mainly high molecular weight, cross-linked materials while the predominant materials in the untreated fibrils (sample 4) contained non-cross-linked collagen $\alpha$-chain (referring to Fig. 2, Exhibit C).

C. Experiment 3

BAPN rat skin samples were dispersed in acetic acid (as described in the Kuntz patent). These acid dispersions (or suspensions) were treated with methylene blue in acid (as described in the Kuntz patent and designated as Treated BAPN Skin Suspension), analyzed and compared to untreated acid dispersions (designated as Control BAPN Skin Suspension):

5. Treated BAPN Acid Suspension - BAPN skin suspended in acetic acid and treated with

methylene blue using the method described in the Kuntz patent.

6. Control BAPN Acid Suspension - BAPN Skin Suspended in acetic acid but not treated.

These samples were analyzed by $NaDodSO_4$ gels (referring to Experiment 3, Figs. 3 and 4, Exhibit C) and the results showed that there is essentially no difference between the treated and the non-treated samples.

15. The lower solubility of both the BAPN rat skin cross-linked in accordance with the method of the present invention (sample 1) and the reconstituted BAPN rat skin collagen fibrils (sample 3) compared to the control rat skin (sample 2) and control reconstituted rat skin collagen fibrils (sample 4) indicates the formation of cross-links between collagen fibrils by the present method. Likewise, the similarity of the gels corresponding to sample 5 (Kuntz process) and sample 6 (control) indicates that, contrary to the claims throughout the Kuntz patent, no cross-links are formed as a result of that process. Stated another way, the fact that no cross-links were formed in collagen at acid pH as described in Examples IV and V of the Kuntz patent indicates that the Kuntz process may not be an effective process for cross-linking collagen fibrils.

16. In light of these results indicating that the Kuntz process does not result in the formation of cross-linkages, it is now possible to suggest why the product of the method of the present invention is so resistant to degradation by proteolytic enzymes while Example VII of the Kuntz patent reports that the product of that process is <u>more</u> susceptible to enzymatic degradation upon exposure to light for longer periods of time: specifically, no cross-linking occurs in the process described in Example VII of the Kuntz patent. In keeping with this conclusion, it would appear that it is appropriate to refer to the Kuntz patent as a method of treating acid-extracted collagen molecules which increases such physical parameters as the tensile strength of the resulting product as well as the susceptibility of that product to enzymatic degradation rather that a method of

forming "thermostable, irreversibly cross-linked collagenous polymers" (quoting from col. 1, line 65 of that patent). This assessment of that patent is consistent with a phenomenon which is well-known to occur with proteinaceous materials, namely, that hydrophobic interactions can occur when heated that change the physical properties of the proteinaceous material without formation of cross-linkages.

17. In summary, it is stated at page 4 of the Official Action of June 27, 1991 that no unexpected results are seen from the use of one form of collagen (i.e., soluble collagen in Kuntz) as compared to another (i.e., native fibrils in the case of the present invention). However, the fact that the results obtained from the use of intact fibrils which have the identical structural conformation as those in native tissue are exactly the opposite of the results obtained by the process described in the Kuntz patent is exactly the kind of difference that is unexpected. Further, the experiments described herein go beyond that difference, demonstrating the unprecedented performance of a bioprosthesis fabricated with the product of the present invention. Finally, the experiments reported herein indicate that the Kuntz patent does not result in the formation of cross-links at all such that, rather than having demonstrated a difference in the type of collagen being cross-linked, these data demonstrate that the Kuntz patent simply is not directed to a method which produces a product which is cross-linked. In my estimation, it is hard to imagine a more surprising set of results. In another word, when one follows the method described in the Kuntz patent, one cannot obtain the stable, cross-linked collagen matrix which is obtained by the method described in the present application.

I hereby declare that all statements made herein of my own knowledge are true and the all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that

such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Oct. 10, 1991
Date

David T. Cheung
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